

# Urea Versus True Protein as Supplement for Lactating Dairy Cows Fed Grain Plus Mixtures of Alfalfa and Corn Silages<sup>1</sup>

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## ABSTRACT

In trial 1, 12 cows averaging 36 kg/d of milk were fed 15.4 to 15.7% CP diets containing 30% corn silage, 26% alfalfa silage with 60% DM, and 32 to 40% corn grain. Four CP supplements were fed in 4 × 4 Latin squares: 1) 1.5% urea, 2) 9.3% soybean meal, 3) 8.2% meat and bone meal, or 4) 4.7% soybean meal and 4.1% meat and bone meal. Except for greater BW gain and lower milk protein on diet 3, production traits did not differ. True protein in the diet lowered plasma urea but did not alter essential AA in plasma. In trial 2, 16 cows averaging 38 kg/d of milk were fed 16.2 to 16.4% CP diets containing 27% corn silage, 27% alfalfa silage with either 39 or 59% DM, and 35 to 43% corn grain. Diets fed in 4 × 4 Latin squares were the following: 1) 1.8% urea, or 2) 5.5% soybean meal plus 5.1% meat and bone meal, fed with 39% DM alfalfa silage; or 3) 1.8% urea, or 4) 5.4% soybean meal plus 5.0% meat and bone meal, fed with 59% DM alfalfa silage. The DMI was greater on diets 2, 3, and 4; BW gain was greater with diets 2 and 4. Yields of milk and milk components were greatest on diet 2 and greater on diet 2 than on diet 1. Yields of milk and protein were not different between diets 3 and 4, but yields of fat and FCM were greater on diet 4 than on diet 3. Lower ruminal ammonia and urea in milk and blood were consistent with lower degradability of the protein meals. Results indicated that dietary true protein increased milk yield when low, but not high, DM alfalfa silage was fed with corn silage as half of the forage.

(Key words: alfalfa silage, corn silage, milk yield, protein utilization)

Abbreviation key: AP = absorbed protein, HDM = high DM alfalfa silage, LDM = low

DM alfalfa silage, MBM = meat and bone meal, SBM = soybean meal, UIP = undegraded intake protein.

## INTRODUCTION

Alfalfa represents a major protein source for lactating cows. However, experimental evidence (5) indicates that excessive rumen degradation of alfalfa protein results in inefficient utilization and depressed yields of milk and milk protein. The NPN content of alfalfa silage typically ranges from about 50% (10) to as high as 87% (19) of total N, and the undegraded intake protein (UIP) of alfalfa silage is reported (22) to be only 80% that of alfalfa hay. Recently, increases in yield were significant in lactating cows fed all of their forage as alfalfa silage when solvent soybean meal (SBM) was replaced with high UIP sources, such as expeller SBM (10), roasted soybeans (15), or fish meal (8). Cows fed all alfalfa silage diets yielded more milk and milk protein when they were abomasally infused with casein (14) and when they were fed expeller SBM rather than equal DM from high moisture corn (12).

Corn silage may contain substantially more NE<sub>L</sub> than does alfalfa silage (22). Greater energy content of diets based on corn plus alfalfa silages is expected to increase microbial protein yields (22) relative to that of diets based on alfalfa silage as the sole forage. This difference may explain our finding that cows yielded less milk protein when they were fed all forage as alfalfa silage than when fed a lower protein diet with equal forage DM from corn silage but with supplemental CP from solvent SBM (5). However, NPN in corn silage, as a proportion of total N, is similar to that in alfalfa silage (4), and alfalfa plus corn silage diets may lack sufficient intact protein for maximal protein synthesis in the rumen (1, 26). Merchen and Satter (17) reported greater NAN digestion in the small intestine when

cows were fed alfalfa silage with 66% DM versus alfalfa silage with 29 or 40% DM. Extensive heating in the silo with drier alfalfa silage may reduce ruminal degradation of protein; hence, response to dietary UIP sources could vary with DM content of alfalfa silage.

The objectives of this study were to determine whether milk yield response to proteins differed when supplements were fed as NPN or as degradable or resistant true proteins and to determine whether milk yield response to NPN or true protein differed when alfalfa silage was ensiled with low DM (LDM) or high DM (HDM) contents.

## MATERIALS AND METHODS

### Protein Supplements

Two lots each of solvent SBM and meat and bone meal (MBM) were obtained (Mounds Inc., Middleton, WI), one each for trials 1 and 2. Two subsamples from each lot were analyzed for CP and DM (2) and for proportions of total N present as ADIN (16) and buffer-soluble N (6). Each sample of SBM and MBM also was assayed for fractional rate of protein degradation and proportion escaping the rumen using an inhibitor in vitro system described earlier (7), except that incubations were conducted in 50-ml tubes and were stopped with TCA only. Mean results of these assays are in Table 1.

### Trial 1

Twelve multiparous Holstein cows ( $\bar{X} \pm \text{SE}$ ) of  $619 \pm 18$  kg of BW, parity  $3.5 \pm .3$ , 49

$\pm 6$  DIM, and  $36 \pm 1$  kg/d of milk were blocked into three groups of nearly equal average parity, DIM, and yield; cows within groups were assigned randomly to three  $4 \times 4$  Latin squares. Diets contained (DM basis; Table 2) 26% wilted alfalfa silage and 30% corn silage (Table 3) plus 32 to 40% ground shelled corn; CP sources were included in the diet at the expense of corn. Alfalfa was wilted, second-cutting silage and contained 60% DM when it was fed. Both alfalfa and corn silages were chopped to a theoretical length of 1.0 cm and stored in concrete stave tower silos. The four CP supplements provided an average 4.3 percentage units of CP each and were fed in the Latin squares. Supplements were 1) urea, 2) solvent SBM, 3) MBM, and 4) solvent SBM plus MBM. Diets were fed for 3-wk periods (total 12 wk). The study was designed to test the effects of protein supply. Milk yield responses to postprandial protein infusion occur very rapidly, within 24 h (9). Hence, 1 wk was considered to be adequate for adaptation to protein supplementation; mean yield and intake data from the last 2 wk of each period were analyzed. Milk yield was recorded daily at a.m. and p.m. milkings. Milk samples were collected at two a.m. and p.m. milkings midway through wk 2 and 3 of each period; a composite sample proportional to the amount of milk yielded at each of the four milkings was prepared each week for every cow. Composites were analyzed for fat by infrared analysis (Wisconsin DHI Cooperative, Madison), total protein as Kjeldahl N  $\times 6.38$  (2), and for true protein as TCA-insoluble N  $\times 6.38$  (5). Cows were weighed on 3 consecutive d at the start of the trial and at the end of each period.

TABLE 1. Composition of true protein supplements.<sup>1</sup>

Components	Trial 1		Trial 2	
	SBM	MBM	SBM	MBM
CP, % of DM	48.3	50.6	48.7	49.8
ADIN, % of total N	1.0	4.4	.8	5.8
Buffer-soluble N, % of total N	23.0	13.4	27.7	13.3
Degradation rate ( $k_d$ ), <sup>2</sup> /h	.140	.061	.154	.046
Intercept (B), % of CP	99.7	95.4	99.6	94.0
Estimated escape, <sup>3</sup> % of CP	30	47	27	53

<sup>1</sup>SBM = Soybean meal; MBM = meat and bone meal.

<sup>2</sup>Ruminal degradation rate determined with an inhibitor in vitro system (7).

<sup>3</sup>[% =  $B \times (k_p / (k_p + k_d))$ ] where ruminal passage rate ( $k_p$ ) is assumed to be .06/h (7).

TABLE 2. Composition of diets.<sup>1</sup>

Item	Trial 1				Trial 2			
	Urea	SBM	MBM	SBM + MBM	LDM + Urea	LDM + Prot.	HDM + Urea	HDM + Prot.
	(% DM)							
Alfalfa silage	25.5	25.5	25.5	25.5	25.7	25.8	27.2	27.2
Corn silage	30.1	30.1	30.1	30.1	27.5	27.6	26.9	27.0
Urea	1.5	...	...	...	1.8	...	1.8	...
SBM	...	9.3	...	4.7	...	5.5	...	5.4
MBM	...	...	8.2	4.1	...	5.1	...	5.0
Ground corn grain	39.8	32.3	35.6	34.1	42.8	35.5	42.0	34.9
Rock phosphate	2.2	2.2	...	1.2	1.3	...	1.3	...
Trace-mineralized salt <sup>2</sup>	.5	.5	.5	.5	.5	.5	.5	.5
Sodium sulfate	.3	...	...	...	.3	...	.3	...
Vitamin premix <sup>3</sup>	.05	.05	.05	.05	.05	.05	.05	.05
Chemical composition								
CP	15.4	15.4	15.7	15.6	16.4	16.3	16.3	16.2
NDF	30.3	30.2	30.6	30.9	30.2	30.9	30.4	31.1
ADF	19.7	20.7	20.7	21.1	17.9	18.6	18.9	19.6
NE <sub>L</sub> <sup>4</sup> Mcal/kg	1.62	1.65	1.67	1.66	1.63	1.68	1.63	1.67
UIP <sup>5</sup> % of CP	28.2	34.4	39.6	37.1	25.9	36.5	26.9	37.3

<sup>1</sup>SBM = Soybean meal; MBM = meat and bone meal; LDM = low DM alfalfa silage; HDM = high DM alfalfa silage; Prot. = SBM plus MBM; UIP = undegraded intake protein.

<sup>2</sup>Provided (mg/kg of DM): Mn, 27; Zn, 27; Fe, 17; Cu, 7; I, .40; Se, .30; and Co, .10.

<sup>3</sup>Provided (IU/kg of DM): vitamin A, 1100; vitamin D, 1100; and vitamin E, .11.

<sup>4</sup>Computed from NE<sub>L</sub> contents of silages and NRC (22) tables.

<sup>5</sup>Computed using percentages of ruminal escape as UIP values for SBM and MBM (Table 1) and UIP values for other feed ingredients from NRC [Table 7-3 (22)].

Diets were fed for ad libitum intake as TMR. Feed was offered twice daily at 0800 and 1600 h, and orts were recorded once daily; feed offered was adjusted daily to yield 5% orts. Weekly composites of each TMR, type of orts, and silage were collected from daily samples of about .5 kg and stored at -20°C; weekly samples of each concentrate mix were collected and stored at room temperature (25°C). Silage content of as-fed rations was adjusted at the beginning of each period based on DM determined at 60°C (48 h). Duplicate composites of diet ingredients and TMR from each period were prepared by grinding silage, TMR (dried at 60°C), and concentrate samples through a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Diet ingredients were analyzed for CP, by the Kjeldahl method using a copper digestion catalyst [Kjeltabs; Tecator Inc., Herndon, VA (2)], NDF and ADF (24), and ADIN (16). Silages were analyzed for buffer-soluble N (6) and NPN (19). Samples of TMR and orts were analyzed for DM

(60°C, 48 h); DMI was reported on this basis. The actual proportion of dietary DM from each component was computed from DM determined by toluene distillation (13) and at 105°C (2) for silage and concentrates, respectively. The NE<sub>L</sub> content of the TMR was calculated using the NE<sub>L</sub> of alfalfa silage, computed from NDF (18), and NE<sub>L</sub> reported in NRC (22) tables. Compositions of TMR and silages fed in trial 1 are in Tables 2 and 3, respectively.

Blood samples were taken 4 h after feeding on d 21 of each period from the coccygeal artery or vein of each cow. Blood was heparinized and stored at 2°C for about 2 h, at which time plasma was prepared and deproteinized using 4 volumes of plasma:1 volume of 15% wt/vol 5-sulfosalicylic acid and then stored at -20°C. Deproteinized plasma was analyzed for individual free AA and urea (21).

Data on mean BW change, DMI, milk yield, and free AA in plasma were analyzed as a 4 × 4 Latin square, replicated three times,

TABLE 3. Composition of forages fed during trials 1 and 2.<sup>1</sup>

Components	Trial 1		Trial 2		
	CS	AS	CS	LDM	HDM
DM, %	38.5	59.7	46.8	39.0	58.6
CP, % of DM	9.1	19.2	8.7	19.9	19.4
NDF, % of DM	50.2	48.5	50.1	49.0	48.4
ADF, % of DM	24.0	39.9	24.7	38.0	40.4
ADIN, % of total N	6.6	7.7	7.6	9.1	9.8
NPN, % of total N	46.0	48.6	43.9	53.4	41.3
Buffer-soluble N, <sup>2</sup> % of total N	52.2	54.0	49.9	57.3	50.5
NE <sub>L</sub> , <sup>3</sup> Mcal/kg of DM	1.69	1.28	1.69	1.27	1.28

<sup>1</sup>CS = Corn silage; AS = alfalfa silage; LDM = low DM AS; HDM = high DM AS; TN = total N.

<sup>2</sup>Proportion of total N soluble in McDougall's buffer (6).

<sup>3</sup>Means for NE<sub>L</sub> for CS (22); values for NE<sub>L</sub> for AS computed from NDF using the equation of Mertens (18).

using the general linear models procedure of SAS (25). The model included square, cow within square, period within square, and treatment. Period by treatment interactions were not significant for any variable tested ( $P \geq .25$ ); thus, they were pooled with the residual. When dietary treatment effects were significant ( $P < .05$ ) for yield traits or  $P < .10$  for free AA in plasma, mean separation was by least significant difference.

#### Trial 2

Sixteen multiparous Holstein cows, including 4 fitted with permanent ruminal cannulas, of ( $\bar{X} \pm \text{SE}$ )  $629 \pm 11$  kg of BW, parity  $3.4 \pm .2$ ,  $41 \pm 5$  DIM, and  $38 \pm 3$  kg/d of milk were blocked into four groups of 4 cows each with nearly equal average parity, DIM, and yield. Cows within groups were assigned randomly to four  $4 \times 4$  Latin squares with 3-wk periods. Diets were fed as TMR and contained (DM basis) 27% alfalfa silage and 27% corn silage (Table 3) plus 35 to 43% ground shelled corn. Both alfalfa silages and the corn silage were chopped to a theoretical length of 1.0 cm and stored in concrete stave tower silos. Alfalfa silage was from third cutting and wilted to either 39% DM (LDM) or 59% DM (HDM) when it was fed. The four dietary treatments in the Latin squares (Table 2) were the following: 1) 1.8% urea plus LDM, 2) 5.5% solvent SBM and 5.1% MBM plus LDM, 3) 1.8% urea plus HDM, and 4) 5.4% solvent SBM and 5.0% MBM plus HDM. Supplements each provided an average of 5.2 percentage units of CP.

Periods, measurements of BW, milk yield, and milk composition were as described in trial 1, except that milk was not analyzed for true protein but was analyzed for lactose and urea (6). Preparation of TMR, adjustment of TMR composition, determination of DMI, and sampling and analyses of feed were as described in trial 1.

Blood samples were taken 4 h after feeding on d 21 of each period from each cow, deproteinized, and stored as described in trial 1; deproteinized plasma was analyzed for glucose and urea (6). Free AA in blood plasma were not determined in trial 2. Also on d 21, strained ruminal fluid, taken from the ventral sac at 0 h (just prior to a.m. feeding) and at 1, 2, 3, 4, and 6 h after feeding, was collected by straining ruminal contents through two layers of cheesecloth. The pH was determined immediately; ruminal fluid then was preserved by addition of 1 ml of 50% (vol/vol) sulfuric acid/50 ml of strained ruminal fluid, and samples were stored at  $-20^\circ\text{C}$ . Strained ruminal fluid was thawed and centrifuged at  $30,000 \times g$  for 15 min at  $2^\circ\text{C}$ , and supernatants were analyzed for ammonia and total AA (6).

Data were analyzed as a  $4 \times 4$  Latin square, which was replicated four times for data on DMI, BW change, milk, and blood plasma and once for ruminal data, using the general linear models procedure of SAS (25). The model for the replicated Latin square included square, cow within square, period within square, treatment, and period by treatment interaction; the model for the ruminal Latin square included

only cow, period, and treatment. When period by treatment interactions were not significant ( $P \geq .13$ ), they were pooled with the residual. Period by treatment was significant for DMI ( $P < .001$ ) and efficiency (milk/DMI;  $P = .036$ ); therefore, this interaction was included in the model for those variables. When dietary treatment effects were significant ( $P < .05$ ), mean separation was by least significant difference.

## RESULTS AND DISCUSSION

### Trial 1

Intake of DM and yields of milk and milk components were unaltered by source of supplemental CP (Table 4). Although total and true milk protein concentrations were lower on the MBM diet, yields of total and true protein were not different among diets. The protein equivalent of milk NPN ( $\text{NPN} \times 6.38$ ) ranged from .12 to .14% and was not influenced ( $P = .82$ ) by CP supplement. The BW gain tended to be greater with true protein supplementation; BW change on MBM was greater than that on urea (Table 4).

In vitro analysis (Table 1) indicated that UIP for MBM was about 60% greater than for SBM. If our UIP estimates (Table 2) apply in vivo, then the SBM, MBM, and SBM plus MBM diets provided .24, .43, and .33 kg/d more UIP than did the urea diet at these DMI (Table 4). The lack of response in milk production and composition to true protein suggested that absorbed protein (AP) was not limiting on the urea diet.

Alfalfa silage fed in this trial contained 60% DM (Table 3). High DM and ADIN contents of alfalfa silage are usually associated with extensive heating in the silo (20, 28) and possibly increased ruminal protein escape (17). Yu and Thomas (27) observed decreased N retention when ADIN content of alfalfa silage increased from 10 to 12 and 15% of total N; ADIN was only 7.7% of total N, suggesting that extensive heating did not occur in the silos in trial 1 (20, 28). The NPN concentrations of corn silage and alfalfa silage were typical of both forages (4, 10). Blood urea was lower (3.8 vs. 5.2 mM) when true protein was fed than when the urea diet was fed (Table 4). This finding probably was due to higher rates of

ruminal ammonia formation when urea was fed. Oltner and Wiktorsson (23) suggested that milk urea concentrations  $<5$  mM indicated that ruminally degraded protein was insufficient to match dietary energy. Urea concentrations in blood and milk generally are similar (6, 10, 23). Concentrations of urea  $<4$  mM ( $<11$  mg of N/dl) in blood imply that ruminally degraded protein may have been limiting on the diets containing true protein. The ratios of ruminally available protein to microbial CP synthesis, predicted from  $\text{NE}_L$  intake (22), were 1.10, .96, .90, and .93, respectively, for the urea, SBM, MBM, and SBM plus MBM diets. These estimates were consistent with inadequate ruminally available protein on the latter three diets. Apparent lack of response to true protein may have occurred because UIP was substituted for reduced microbial protein supply in cows fed the urea diet.

Free AA in plasma are in Table 5. The nonessential AA, Pro and Gly, generally were at higher concentrations when SBM and MBM were supplemented alone; total nonessential AA were higher on the MBM diet than on the MBM plus SBM diet. Except for Trp, which was higher, neither individual nor total essential AA were altered when true protein was fed. Because milk protein yield did not differ (Table 4), increased intestinal protein supply should be reflected in elevated concentrations of individual essential AA, particularly branched-chain AA (3, 21). The general lack of response of essential AA in blood plasma indicated that protein status was not improved when dietary true proteins were fed in this trial; reduced ruminal protein degradation may have limited microbial protein synthesis. The  $\text{NE}_L$  content of these TMR probably was sufficient to stimulate utilization of the silage NPN in all diets, plus the additional NPN in the urea diet, and intestinal supply of protein AA may not have differed among the four diets.

### Trial 2

Data on feed DMI, BW change, milk yield, urea in milk and plasma, and glucose in plasma are in Table 4. Intake averaged about 1.9 kg/d of DM less on LDM plus urea diet than on the other three diets; the pattern was the same when DMI was expressed as a percentage of BW. Change in BW and yields of

TABLE 4. Effect of diet on DMI, BW gain, yields of milk and milk components, and concentrations of urea in milk and urea and glucose in plasma (trials 1 and 2).<sup>1</sup>

Item	Trial 1					Trial 2				
	Urea	SBM	MBM	SBM + MBM	SE	P > F <sup>2</sup>	LDM + Urea	LDM + Prot.	HDM + Urea	HDM + Prot.
DMI, kg/d	25.4	25.3	24.7	24.9	.4	.486	24.2 <sup>b</sup>	26.2 <sup>a</sup>	26.0 <sup>a</sup>	26.1 <sup>a</sup>
DMI, % of BW	3.96	3.89	3.78	3.82	.05	.065	3.79 <sup>b</sup>	4.06 <sup>a</sup>	4.09 <sup>a</sup>	4.08 <sup>a</sup>
BW Change, kg/d	.33 <sup>b</sup>	.53 <sup>ab</sup>	1.02 <sup>a</sup>	.79 <sup>ab</sup>	.17	.044	-.25 <sup>b</sup>	.57 <sup>a</sup>	-.01 <sup>b</sup>	.26 <sup>ab</sup>
Milk, kg/d	32.9	32.6	33.4	32.9	.7	.874	35.4 <sup>c</sup>	38.5 <sup>a</sup>	35.9 <sup>bc</sup>	36.9 <sup>b</sup>
3.5% FCM, kg/d	33.1	32.1	33.0	33.3	.9	.797	36.0 <sup>bc</sup>	38.2 <sup>a</sup>	35.7 <sup>c</sup>	37.1 <sup>ab</sup>
Fat %	3.59	3.44	3.44	3.57	.08	.414	3.70	3.48	3.47	3.54
kg/d	1.16	1.11	1.14	1.17	.04	.685	1.28 <sup>ab</sup>	1.33 <sup>a</sup>	1.24 <sup>b</sup>	1.30 <sup>a</sup>
Total protein %	3.23 <sup>a</sup>	3.27 <sup>a</sup>	3.14 <sup>b</sup>	3.25 <sup>a</sup>	.03	.030	3.09	3.01	3.04	3.02
kg/d	1.05	1.06	1.04	1.06	.02	.792	1.09 <sup>b</sup>	1.15 <sup>a</sup>	1.09 <sup>b</sup>	1.11 <sup>ab</sup>
True protein %	3.09 <sup>ab</sup>	3.14 <sup>a</sup>	3.01 <sup>b</sup>	3.13 <sup>a</sup>	.03	.022	ND	ND	ND	ND
kg/d	1.00	1.02	.99	1.02	.02	.731	ND	ND	ND	ND
Lactose %	ND	ND	ND	ND	...	...	4.98	5.03	4.99	5.00
kg/d	ND	ND	ND	ND	...	...	1.77 <sup>c</sup>	1.94 <sup>a</sup>	1.80 <sup>bc</sup>	1.85 <sup>b</sup>
Efficiency, milk/DMI	1.29	1.29	1.35	1.33	.04	.619	1.47 <sup>a</sup>	1.47 <sup>a</sup>	1.38 <sup>b</sup>	1.42 <sup>b</sup>
Urea in milk, mM	ND	ND	ND	ND	...	...	6.97 <sup>a</sup>	4.79 <sup>b</sup>	7.06 <sup>a</sup>	4.54 <sup>b</sup>
Urea in plasma, mM	5.22 <sup>a</sup>	3.81 <sup>b</sup>	3.91 <sup>b</sup>	3.74 <sup>b</sup>	.27	.002	6.57 <sup>a</sup>	4.70 <sup>b</sup>	6.68 <sup>a</sup>	4.65 <sup>b</sup>
Glucose in plasma, mg/dl	ND	ND	ND	ND	...	...	62.2	60.0	59.0	60.6

<sup>a,b,c</sup>Means within trial with no common superscripts differ ( $P < .05$ ).<sup>1</sup>SBM = Soybean meal; MBM = meat and bone meal; LDM = low DM alfalfa silage; HDM = high DM alfalfa silage; Prot. = SBM plus MBM; ND = not determined.<sup>2</sup>Probability of a significant dietary treatment effect.

milk, FCM, fat, protein, and lactose were highest on the LDM plus protein diet; most yield traits were intermediate on the HDM plus protein diet and lowest on the two diets containing urea. Concentrations of fat, protein, and lactose in milk were not affected by diet.

With true protein supplementation, yield on the LDM diet exceeded that on the HDM diet (at equal DMI). Yields of milk and milk components were similar on both diets containing urea, despite nearly 2 kg/d greater DMI on the HDM diet. Loss of .25 kg/d of BW with the LDM plus urea diet and gain of .57 kg/d of BW with the LDM plus protein diet are equivalent to a difference in energy requirement of 4.15 Mcal/d of NE<sub>L</sub> (22); NE<sub>L</sub> intake

was 4.57 Mcal/d greater on the LDM plus protein diet. Thus, yield responses in this trial were not due solely to effects on DMI.

Ration UIP estimates (Table 2) and DMI (Table 4) were used to compute in vivo UIP supply; the LDM plus protein and HDM plus protein diets provided .53 and .44 kg/d more UIP. When true protein supplemented LDM, increased yields of milk, protein, and lactose indicated that cows were responsive to added UIP. Except for FCM and fat, yields were similar with HDM regardless of CP supplement, indicating that additional UIP was not effective. Similar ADIN contents in LDM and HDM (Table 3) indicated that overheating did not occur in either silage (27, 28) and sug-

TABLE 5. Concentrations of protein AA and 3-methyl His in blood plasma of cows fed differing CP supplements in trial 1.<sup>1</sup>

AA	Urea	SBM	MBM	SBM + MBM	SE	P > F <sup>2</sup>
	(nmol/ml)					
Thr	99.5	110.8	103.0	97.4	4.6	.209
Ser	90.4 <sup>b</sup>	96.7 <sup>ab</sup>	109.9 <sup>a</sup>	90.8 <sup>b</sup>	5.0	.037
Asp	12.2	12.5	13.7	12.0	.8	.424
Asn	56.7	59.1	56.1	55.8	3.1	.869
Glu	127.7	118.4	122.0	115.5	7.4	.683
Gln	140.0	123.1	129.0	123.8	15.0	.846
Pro	95.3 <sup>b</sup>	99.8 <sup>ab</sup>	117.3 <sup>a</sup>	94.6 <sup>b</sup>	6.1	.047
Gly	353.6 <sup>b</sup>	361.1 <sup>ab</sup>	425.8 <sup>a</sup>	350.0 <sup>b</sup>	23.4	.099
Ala	123.3	132.5	132.2	123.9	4.4	.288
Val	231.6	259.9	268.7	249.4	13.6	.273
Cys	41.3	43.6	38.0	40.9	2.8	.576
Met	19.8	19.2	20.0	19.8	1.7	.988
Ile	94.1	110.2	102.7	99.5	5.6	.254
Leu	143.3	161.5	169.4	153.2	8.3	.170
Tyr	55.1	58.5	55.6	54.6	3.1	.820
Phe	41.9	46.6	46.2	44.3	2.2	.427
Trp	21.7 <sup>b</sup>	31.1 <sup>a</sup>	29.9 <sup>a</sup>	26.8 <sup>ab</sup>	2.7	.092
Lys	72.4	84.3	79.1	79.5	5.0	.434
His	52.3	55.0	55.6	53.9	3.3	.900
Arg	62.0	67.2	72.2	64.0	3.5	.220
3-Methyl-His	5.7 <sup>a</sup>	5.2 <sup>ab</sup>	5.8 <sup>a</sup>	4.2 <sup>b</sup>	.4	.021
BCAA <sup>3</sup>	469	532	541	502	27	.243
EAA <sup>4</sup>	935	1048	1040	983	46	.294
NEAA <sup>5</sup>	999 <sup>ab</sup>	1003 <sup>ab</sup>	1106 <sup>a</sup>	966 <sup>b</sup>	38	.080
BCAA/Gly	1.40	1.56	1.30	1.48	.09	.246
EAA/NEAA	.94	1.05	.95	1.03	.04	.114

<sup>a,b</sup>Means with no common superscripts differ ( $P < .10$ ).

<sup>1</sup>SBM = Soybean meal; MBM = meat and bone meal.

<sup>2</sup>Probability of a significant dietary treatment effect.

<sup>3</sup>Branched-chain AA: sum of Val, Ile, and Leu.

<sup>4</sup>Essential and semiessential AA: sum of Thr, Val, Cys, Met, Ile, Leu, Tyr, Phe, Trp, Lys, His, and Arg.

<sup>5</sup>Nonessential AA: sum of Ser, Asp, Asn, Glu, Gln, Pro, Gly, and Ala.

TABLE 6. Effect of source of supplemental CP and alfalfa silage DM on pH and concentration of N metabolites in ruminal fluid (trial 2).<sup>1</sup>

Item	LDM + Urea	LDM + Prot.	HDM + Urea	HDM + Prot.	SE	<i>P</i> > <i>F</i> <sup>2</sup>
pH	6.57 <sup>a</sup>	6.30 <sup>b</sup>	6.62 <sup>a</sup>	6.49 <sup>a</sup>	.04	.014
Ammonia, mM	20.9 <sup>a</sup>	11.3 <sup>b</sup>	18.9 <sup>a</sup>	12.1 <sup>b</sup>	1.34	.009
Total AA, mM	1.40	1.72	.87	1.71	.35	.386

<sup>a,b</sup>Means with no common superscripts differ ( $P < .05$ ).

<sup>1</sup>LDM = Low DM alfalfa silage; HDM = high DM alfalfa silage; Prot. = soybean meal plus meat and bone meal.

<sup>2</sup>Probability of a significant dietary treatment effect.

gested that the extensive additional heating expected with HDM had not occurred. Merchen and Satter (17) reported that the amount of NAN apparently digested in the small intestine, as a proportion of N intake, increased 35% when cows were fed HDM with 66% DM versus LDM with 29 or 40% DM. However, Broderick et al. (11) observed that yields of milk and milk components in cows fed LDM with 36% DM were greater than that on HDM with 61% DM. We interpret the present results to mean that nutrients in LDM were utilized better than those in HDM when the other half of dietary forage came from corn silage.

Supplies of AP [computed from UIP (Table 2), microbial protein synthesis (estimated from  $NE_L$  intake), and the AP required for, or mobilized from, BW change (22)] were 2.5, 2.9, 2.7, and 3.0 kg/d, for the LDM plus urea, LDM plus protein, HDM plus urea, and HDM plus protein diets, respectively. Milk yields predicted from AP and  $NE_L$  supplies on these diets [including estimates of  $NE_L$  mobilized from tissue losses or required for BW gain (22)] were, respectively, 36 and 45 kg/d (LDM plus urea), 45 and 46 kg/d (LDM plus protein), 40 and 47 kg/d (HDM plus urea), and 46 and 47 kg/d (HDM plus protein). Compared with actual milk yields (Table 4), these estimates suggest that AP supply limited milk yield only on the LDM plus urea diet. Because  $NE_L$  intake on all diets was sufficient for yields of 45 to 47 kg/d, true protein, HDM, or both appeared to correct an inadequate AP supply on the LDM plus urea diet. Milk yield on the other three diets was about 4 to 9 kg/d less than predicted based on AP supply and 7 to 12 kg/d less than predicted based on  $NE_L$  supply. Other environmental factors or genetics may

have limited milk yield in this trial to a maximum of 38 to 39 kg/d.

Urea concentrations in milk and blood plasma (Table 4) were of similar magnitude and pattern; they were higher on both urea-supplemented diets (ranging from 6.6 to 7.1 mM) and lower on the true protein-supplemented diets (ranging from 4.5 to 4.8 mM). Effect of diet on glucose in blood was not significant. Patterns of ruminal ammonia concentration and pH were similar to those of urea in milk and blood, higher on urea than true protein-supplemented diets (Table 6). This finding confirms the usefulness of urea in milk and urea in blood as indices of ruminal ammonia concentrations at similar CP percentages in the diet (6, 10, 23). The higher ruminal pH reflected elevated ammonia in ruminal fluid on diets containing urea. The elevation in total AA in the rumen when true protein was fed was not significant.

## CONCLUSIONS

The usefulness of supplemental CP from urea or true protein differed according to milk yields, DMI, and alfalfa silage DM content in cows fed concentrate and alfalfa plus corn silages. In trial 1, alfalfa silage contained 60% DM; cows averaged 33 kg/d of milk and 25.1 kg/d of DMI. Yields of milk and milk components and concentrations of free AA in plasma were similar when cows were supplemented with CP from urea, SBM, MBM, or SBM plus MBM. In trial 2, cows averaged 37 kg/d of milk and 25.6 kg/d of DMI. Compared with urea, SBM plus MBM with LDM at 39% DM increased yield of milk and milk components. Yields of milk, protein, and lactose did not



differ when either urea or SBM plus MBM were fed with HDM at 59% DM. Lower concentrations of ruminal ammonia and of urea in milk and blood indicated lower degradability of the true proteins. Results indicated that supplementation of true protein increased milk yield when LDM, but not HDM, was fed with corn silage as half of the TMR forage.

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